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ORIGINAL INVESTIGATION

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Modulation of nicotine-induced attentional enhancement in rats by adrenoceptor antagonists

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Abstract *Rationale:* Understanding the neuropharmacological mechanisms mediating attentional enhancement by nicotine would help a targeted search for nicotinic compounds with retained therapeutic but reduced unwanted side-effects. Previous studies suggested that the dopamine-releasing effects of nicotine may not be of primary importance for its attention-enhancing properties. *Objectives:* The present study examined the role of noradrenergic neurotransmission for the effects of nicotine on different response indices of an attentional paradigm. *Methods:* The effects of systemic injections of the α_1-adrenoceptor antagonist prazosin that also displays significant affinity at α_{2B} and α_{2C}-adrenoceptors and the β-adrenoceptor antagonist propranolol were tested in both the presence and absence of nicotine in rats trained in a version of the five-choice serial reaction time task. *Results:* Nicotine generally enhanced the accuracy of signal detection, reduced omission errors and shortened response latencies. At the largest doses tested, both prazosin (1 mg/kg) and propranolol (10 mg/kg) impaired performance. For propranolol, these effects depended on the rate of target signal presentation. The two compounds differentially modulated the effects of nicotine. Propranolol (6 mg/kg and 10 mg/kg) but not prazosin reduced its effects on omission errors and accuracy. By contrast, prazosin (0.5 mg/kg) reversed the nicotine-induced reductions in response latency. *Conclusions:* The data provide the first evidence that β-adrenoceptors are involved in mediating the effects of nicotine on signal detection, while activation of α-adrenoceptors may contribute to its effects on response speed. This is a further indication that, from among nicotine's wide range of neuropharmacological effects, specific facets can be dissociated that are responsible for its attention-enhancing properties.

Keywords Nicotine · Attention · Serial reaction time · Noradrenaline · Prazosin · Propranolol

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Introduction

Nicotine has a broad range of centrally mediated behavioural effects, acting through different subtypes of the neuronal nicotinic acetylcholine receptor (Gotti et al. 1997; Clementi et al. 2000). Via presynaptic and postsynaptic mechanisms, it stimulates the release of various neurotransmitters including acetylcholine, dopamine, noradrenaline, serotonin, glutamate and GABA (Summers and Giacobini 1995; Wonnacott 1997). Knowing which events down-stream from nicotinic receptor activation are critical for specific behavioural effects would enable a targeted search for more selective nicotinic agonists with a narrower behavioural profile. Thus, it may be possible to separate potentially therapeutic effects of nicotine such as attentional enhancement from undesirable effects such as its psychomotor stimulant or addictive properties.

Nicotine-induced attentional enhancement has been well documented in humans (e.g. Koelega 1993; Foulds et al. 1996; Levin et al. 1998). Recent studies in rats demonstrated that nicotine reliably increased the accuracy of signal detection in the five-choice serial reaction time task (5-CSRTT), where light stimuli are presented randomly in one of five locations (Hahn et al. 2002a; Hahn and Stolerman 2002). This was strongly indicative of effects in rodents reflecting attention-enhancing properties of nicotine, which facilitated their pharmacological characterisation. For example, a D_2-antagonist reversed nicotine-induced reductions in response latency, but neither D_1-antagonism nor D_2-antagonism affected the

increase in stimulus detection (Hahn et al. 2002b). Isoarecolone, a nicotinic agonist with a weaker dopamine-releasing action, had no effect on response latency but improved accuracy (Hahn et al. 2003a). Thus, different neuropharmacological effects of nicotine seem responsible for its speed-enhancing and attention-enhancing effects, with those mediating attentional enhancement remaining to be established.

The present study investigated the role of adrenoceptors for the effects of nicotine on attention. The major grouping of central noradrenergic (NA) nuclei in the pons and medulla is the locus coeruleus (LC) from where projections diverge onto multiple brain areas. Systemically administered nicotine stimulates noradrenaline (NA) release in the cortex (Summers and Giacobini 1995), hypothalamus (Sharp and Matta 1993), hippocampus and amygdala (Fu et al. 1998). This effect is mediated by nicotinic receptors located both in the LC and on NA nerve terminals (Mitchell 1993). There are three subclasses of adrenoceptors (α_1, α_2 and β), each with three known subtypes (α_{1A}, α_{1B}, α_{1D}; $\alpha_{2A/D}$, α_{2B}, α_{2C}; β_1, β_2, β_3), and all except β_3 are expressed in rat CNS (Nicholas et al. 1996). Inhibitory autoreceptors in the CNS appear to be of the $\alpha_{2A/D}$-subtype (Raiteri et al. 1992; Norenberg et al. 1997; Ho et al. 1998).

Effects of modulating NA neurotransmission were examined previously in the 5-CSRTT. An α_1-adrenoceptor agonist and antagonist enhanced and impaired stimulus detection, respectively (Puumala et al. 1997), while an α_2-antagonist enhanced it under certain task conditions, presumably by stimulating NA release via autoreceptor blockade (Sirvio et al. 1993). Effects of selective β-adrenoceptor ligands have not been investigated in this paradigm. Rats with lesions of the dorsal NA bundle showed attentional deficits under non-specific behavioural activation (Carli et al. 1983; Cole and Robbins 1987, 1992). This was interpreted as impairment in accurate orienting to the target stimuli, or in non-automatic behavioural organisation necessary for maintaining attentional selectivity under such conditions. A role of NA in adapting to new behavioural demands was suggested by enhanced LC firing or prefrontal NA efflux in response to changing behavioural contingencies (Sara and Segal 1991; Dalley et al. 2001).

Electrophysiological studies supported a role of the LC in regulating attentiveness (e.g. Aston-Jones and Bloom 1981; Foote et al. 1991). Phasic activation of LC neurons was elicited by stimuli that were physically salient, unpredictable or behaviourally significant, highest discharge rates being accompanied by behavioural orienting (Aston-Jones et al. 1991). In monkeys performing a visual discrimination task, LC neurons were selectively activated by target stimuli, and robust LC responsiveness was associated with high discrimination performance. Neuronal and behavioural response latencies correlated on a trial by trial basis (Aston-Jones et al. 1994). An optimal range of NA levels may exist since both very high and low tonic LC activity weakened phasic responsiveness to targets and increased false alarms (Usher et al. 1999).

Studies suggesting that central NA modulates distractibility and rapid reorienting of attention (Coull 1994; Coull et al. 2001) would be in accordance with NA mediation of the effects of nicotine in the 5-CSRTT. These were particularly pronounced in the presence of salient visual or auditory distractors, suggesting improvements in selective or rapid reorienting of attention (Hahn et al. 2002a; Hahn and Stolerman 2002). The present study examined modulation of the effects of nicotine in the 5-CSRTT by prazosin and propranolol, competitive antagonists at α_1-adrenoceptors and β-adrenoceptors, respectively, that readily enter the CNS and do not differentiate between subtypes of α_1-adrenoceptors or β-adrenoceptors. Prazosin displays significant affinity also at the α_{2B} and α_{2C} subtypes, but not at $\alpha_{2A/D}$, thus sparing autoreceptors (Bylund et al. 1992; Ordway et al. 1993).

Materials and methods

Subjects

Male hooded Lister rats (Harlan Olac, Bicester, UK) weighing at least 300 g at the beginning of training were housed individually in a temperature (20±1°C) and humidity (50±10%) controlled environment, on a 12 h light–dark cycle with lights on at 7 a.m. Rats had free access to water and were placed on a food-restricted diet at the beginning of training to maintain them at 85% of their free-feeding weights. The treatment of animals complied with British Law, the Code of Practice of the Institute of Psychiatry and the "Principles of laboratory animal care" (http://www.nap.edu/readingroom/books/labrats/).

Apparatus

Aluminium operant conditioning chambers measuring 26×26×26 cm^3 (Paul Fray Ltd, Cambridge, UK) were housed in sound-insulated and ventilated enclosures. The curved rear wall of each chamber contained five 2.5 cm square holes, 5 cm deep and 5 cm above floor level. At the entrance of each hole, a photocell monitored interruptions of a beam of infrared light, and at the rear there was a green light-emitting diode. A food tray, the entrance to which was covered by a hinged flap, was located in the opposite wall, equidistant from each aperture. Illumination of each chamber was provided by a houselight situated in its roof. The apparatus and data collection were controlled by software running under RISC OS on an Acorn computer in an adjoining room.

Behavioural procedure

The training procedure was similar to that described by Mirza and Stolerman (1998). In the final form of the task, light stimuli of 1 s duration were presented randomly in one of the holes after an intertrial interval (ITI) of 5 s. If the subject nose-poked into the hole while it was illuminated or within 5 s after the light had terminated (limited hold), a 45 mg food pellet was delivered into the food tray and a correct response was registered. A response into any other hole was recorded as an incorrect response and resulted in a 2 s time-out, during which the house light was extinguished. A failure to respond before the end of the limited hold was registered as an omission error. A new trial began with the automatic initiation of an ITI by a correct response, or by time-outs or limited holds in cases of incorrect responses or omission errors. Responses during ITIs had no programmed consequences and were recorded as anticipatory

responses. All training and test sessions lasted 30 min. Rats were trained for 4 months and tests started when stable performance of <20% omissions and >70% correct responses was acquired.

In an effort to maximize scientific gain per laboratory animal and to reduce the number of animals used, all rats had been subjects in previous experiments involving infrequent (at most twice a week) drug administration, with a maximum of eight administrations in total. Experiments 1a and b were conducted in one group of 18 rats that had been exposed to nicotine and the nicotinic antagonist dihydro-β-erythroidine. Experiments 2–6 were conducted in another group of 22 rats that had received either raclopride or SCH23390 and nicotine in a previous experiment (Hahn et al. 2002b). Prior to the start of the experiments reported here, rats had been drug-free and trained for 3 weeks (first group, n=18) and 6 weeks (second group, n=22). One rat did not complete experiment 1b due to a paw injury and was excluded from analyses, resulting in n=17. One rat was excluded from analysis of experiment 4 because it displayed unstable performance on training days intervening test days, and one rat was put down prior to experiment 6 due to a veterinary condition, resulting in n=21 for experiments 4 and 6.

In the training period following experiment 4, in order to avoid ceiling effects in further experiments, the stimulus duration was individually adjusted so that rats typically earned between 130 and 170 reward pellets per session. Titration and stabilization of performance on the new parameters took 1 month, at the end of which stimulus durations ranged from 0.3 s to 1 s across rats. Stimulus durations were kept constant for each rat after this period.

The version of the 5-CSRTT employed in the current and in several previous studies (Hahn et al. 2002a,b, 2003a,b, Hahn and Stolerman 2002) differed from the original version as described by Carli et al. (1983). Omission errors and anticipatory responses were not punished, trials were initiated automatically, not by a panel push by the subject, and the session length was independent of the number of trials completed. The rationale for most of the changes was to reduce the number of different behavioural contingencies controlling performance and to create greater focus on stimulus detection demands. The significance of the changes is further discussed in Hahn et al. (2002a).

Task performance was reflected in the following behavioural measures that were recorded within each session for three successive time periods of 10 min each.

Percentage of correct responses (accuracy): 100×[correct responses/(correct+incorrect responses)]. Accuracy was not calculated when fewer than ten responses had been emitted. This is a measure of response choice. It is calculated on the basis of responses that have been emitted and is not influenced by the rate or speed of responding, thus representing the main index of stimulus detection and attentional performance.

Percentage of omission errors: 100×(omission errors/stimuli presented). Omission errors are influenced by stimulus detection but also by the general rate of responding. It is therefore not a pure measure of attentional performance, although affected by it.

Latency of correct responses: the mean time between stimulus onset and a nose-poke in the correct hole. The latency was not determined if less than five responses had been emitted. It reflects the speed of visual information processing and of initiating and executing the motor response.

Anticipatory response rate: (number of responses in ITIs/number of trials)/ITI-length (s). This yields the number of responses emitted per second, averaged across trials. Anticipatory responses have no direct influence on reward payoff and should essentially be uninhibited. They can reflect rate-increasing or rate-decreasing effects on non-contingent responding but appear to be modulated also by motivational processes (Blondel et al. 2000; Bizarro and Stolerman 2003).

Experimental design

Experimental test sessions were conducted on Tuesdays and Fridays with training sessions on all other weekdays. In test sessions of experiments that involved nicotine administration, the ITI was set to 15 s, as opposed to 5 s in training sessions. Performance enhancing effects of nicotine were previously found to occur reliably under these conditions (Hahn et al. 2002a,b). Experiments that did not involve nicotine administration (experiments 2 and 5) were performed with ITI 5 s, as during training.

Prazosin was administered subcutaneously (SC) and propranolol intraperitoneally (IP) 30 min before test sessions. Nicotine was injected SC 10 min before test sessions. In experiments that involved the administration of prazosin or propranolol and nicotine, each rat had two test sessions with each dose of the respective antagonist, i.e. one in the presence and one in the absence of nicotine. Within each experiment, all treatment conditions were tested in a sequence that was randomised for each individual rat. Experiments were separated by training periods of 2–3 weeks, during which no drugs were given and rats performed the 5-CSRTT only on training parameters.

Drugs

(−)-Nicotine bitartrate (BDH, Poole, UK) was dissolved in isotonic saline, and the pH was adjusted to 7 with NaOH solution. Prazosin hydrochloride and (±)-propranolol hydrochloride (Sigma, Dorset, UK) were dissolved in distilled water. Prazosin was injected at a volume of 2 ml/kg; all other injections were given at a volume of 1 ml/kg. Subcutaneous injections were given into the flank. All doses are expressed as those of the base.

Data analysis

Each of the four measures was analysed separately by two-factor and three-factor ANOVA for repeated measures, followed by one-factor ANOVA, Dunnett's tests and paired t-tests. P-values from t-tests were subjected to Bonferroni correction according to the number of t-tests performed per variable (four in experiments 1a and 3, three in experiment 4, and two in experiments 1b, 5 and 6). Percentage data were arc-sine transformed, latency data were log transformed and anticipatory response data were subject to square root transformation. The purpose was to maximize normal distribution and homogeneity of variances for statistical analyses. In the figures, results are presented as raw values. It is therefore important to note that the data shown in the figures do not directly align with statistical analyses, including results of t-tests and Dunnett's tests that are indicated on the figures. Analyses of data from the first one or two experiments with each antagonist included time period as a factor in order to examine whether the effects of the drugs were stable over the course of the sessions. All analyses were carried out using Unistat 5.0 (Unistat Ltd, London, UK).

Results

Experiment 1a: dose–response study with prazosin

Prazosin (0.0, 0.1, 0.3 and 1 mg/kg) was tested in the presence of nicotine (0.1 mg/kg) or vehicle over a sequence of eight test sessions. In three-factor ANOVA with prazosin, nicotine and time period as within-subject factors nicotine had significant main effects on response accuracy, omission errors and response latency [$F(1,17)$ >6.73, P<0.02 for all three variables], but not on anticipatory responding. These indicated that nicotine enhanced accuracy and reduced omissions and latency of responding across all doses of prazosin. Significant effects of nicotine in the absence of prazosin, however, occurred

only on omission errors (see Fig. 1). Thus, on most measures, modulation of these effects by prazosin could not be investigated in this experiment.

Despite the lack of effect of nicotine alone, the nicotine×prazosin interaction only narrowly failed significance on response accuracy [$F(3,51)=2.66$, $P=0.052$] and was highly significant on anticipatory responding [$F(3,51)=8.82$, $P<0.001$]. On both measures (compare Fig. 1a and d), effects of nicotine were seen only in the presence of the largest dose of prazosin as determined by paired *t*-tests. They were opposite in direction with nicotine enhancing accuracy and reducing anticipatory responding. An inverse correlation between these two measures was previously reported (Hahn et al. 2002a), and the possibility of a causal relationship will be elaborated in the discussion.

Also on omission errors (Fig. 1b), the effects of nicotine interacted with those of prazosin [$F(3,51)=5.52$, $P<0.01$]. One-factor ANOVA yielded a significant effect of prazosin in the presence of nicotine [$F(3,51)=13.59$, $P<0.001$] but not in its absence. Thus, prazosin increased omissions to a larger extent when tested against nicotine. On response latency (Fig. 1c), the increase by prazosin gave rise to a significant main effect [$F(3,51)=19.9$, $P<0.001$] but did not interact with nicotine.

The effects of nicotine interacted with time period only on anticipatory responding [$F(2,34)=6.53$, $P<0.01$], where its depressant effects were most pronounced in the first 10 min of the session. The effects of prazosin interacted with time period only on omission errors [$F(6,102)=2.43$, $P<0.05$]; the increase by its largest dose was strongest in the first period (data not shown).

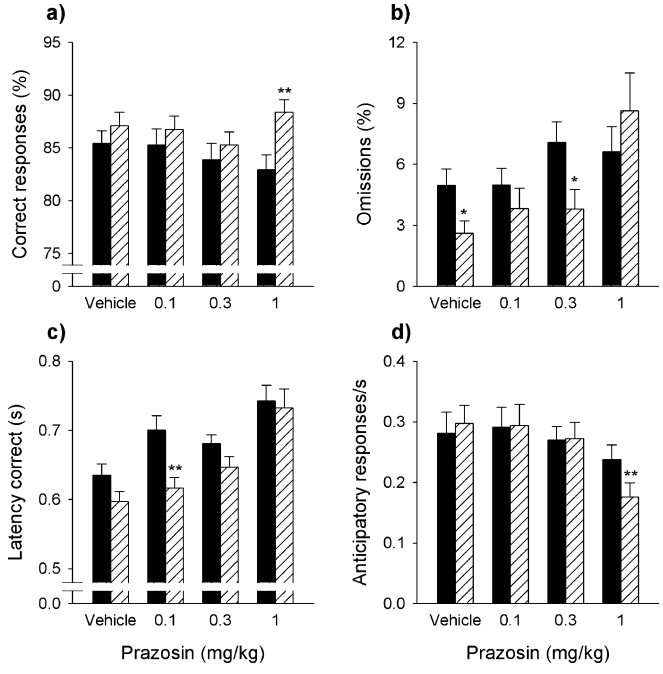

Fig. 1 The effects of systemic injections of prazosin on performance after vehicle (*black bars*) and 0.1 mg/kg of nicotine (*hatched bars*). *Bars* represent the mean performance (±SEM) of 18 rats in 30-min test sessions with ITI 15 s. Please note axis breaks in **a**,**c**. Conditions where nicotine produced a significant difference compared to vehicle are marked (*adjusted $P<0.05$, **adjusted $P<0.01$; paired *t*-tests)

Experiment 1b: supplementary study with one dose of prazosin

In order to test for a possible interaction of nicotine and prazosin on response accuracy that was indicated by a trend in experiment 1a, the largest dose of prazosin (1 mg/kg) and the vehicle control were re-tested in the same group of rats in the presence of nicotine (0.1 mg/kg) or vehicle over a sequence of four tests. Data were combined with those from matching treatment conditions of experiment 1a to increase statistical power.

The effects of prazosin now interacted with those of nicotine on accuracy, omission errors and anticipatory responding [$F(1,16)>5.15$, $P<0.05$ for all three variables] in three-factor ANOVA with nicotine, prazosin and experiment (a versus b) as within-subject factors. As in experiment 1a, nicotine improved accuracy only in the presence of prazosin [$t(33)=4.82$, adjusted $P<0.001$, paired *t*-test], which appeared to impair it when given alone. Similarly, on anticipatory responding, nicotine only had an effect in the presence of prazosin [$t(33)=4.66$, adjusted $P<0.001$], where it appeared to potentiate response-depression by prazosin alone. By contrast, the reduction in omission errors by nicotine alone [$t(33)=4.66$, adjusted $P<0.001$] was not seen in the presence of prazosin. Nicotine now significantly reduced response latency in the absence [$t(33)=4.05$, adjusted $P<0.001$] but not in the presence of prazosin, but there was no significant interaction in ANOVA. The increase in latency by prazosin alone may have prevented such interaction. This response-slowing by prazosin interacted with experiment [$F(1,16)=10.2$, $P<0.01$]; it was larger in experiment 1a (~100 ms) than 1b (~50 ms). Drug effects did not differ between experiments on any other measure.

Experiment 2: dose–response study with propranolol alone

Propranolol (0.0, 2.5, 5 and 10 mg/kg) was tested over four sessions under training parameters (ITI 5 s) to establish dose–response curves for later interaction studies. This and all subsequent experiments were performed in a different group of rats than experiment 1.

Figure 2 illustrates that propranolol reduced accuracy and increased omission errors, response latency and anticipatory responding. This was confirmed by highly significant main effects of propranolol in two-factor ANOVA with propranolol and time period as within-subject factors [$F(3,63)>7.81$, $P<0.001$ for all variables except latency where $F(3,63)=5.22$, $P<0.01$]. The effects of propranolol interacted with time period on accuracy, latency and anticipatory responding [$F(6,126)>2.67$, $P<0.05$ for all three variables]. The effects of propranolol on accuracy and latency weakened with time on task due

to baseline shifts into the same direction as the effects of the drug. On anticipatory responding, its effects increased with time due to shifts in baseline performance in the opposite direction as the effects of propranolol (data not shown).

Experiment 3: dose–response study with propranolol and nicotine

Propranolol (0.0, 1.5, 3 and 6 mg/kg) was tested in the presence and absence of nicotine (0.1 mg/kg) under ITI 15 s over a sequence of eight test sessions. For this interaction study, a maximum dose of 6 mg/kg of propranolol was chosen in order to minimize effects on baseline performance.

As can be seen from Fig. 3, nicotine enhanced the accuracy of responding and reduced omission errors, response latency and anticipatory responding. This was supported by highly significant main effects of nicotine [$F(1,21)>44.1$, $P<0.001$ for all variables except anticipatory responding where $F(1,21)=10.1$, $P<0.01$] in three-factor ANOVA with nicotine, propranolol and time period as within-subject factors.

A significant main effect of propranolol occurred only on response latency [$F(3,63)=5.15$, $P<0.01$], probably reflecting the reduction at the smallest dose when tested alone (Fig. 3c). The otherwise complete absence of effect by propranolol alone was surprising in view of the impairments observed under training parameters in experiment 2. However, Fig. 3b suggests that the largest tested dose of propranolol antagonised the reduction in omission errors by nicotine, which was supported by a significant nicotine×propranolol interaction [$F(3,63)=4.08$, $P=0.01$]. One-factor ANOVA confirmed a significant effect of propranolol in the presence of nicotine [$F(3,63)=4.76$, $P<0.01$] but not when tested against vehicle. Propranolol did not interact with the effects of nicotine on any other measure. Also, none of the interactions involving time period as a factor were significant.

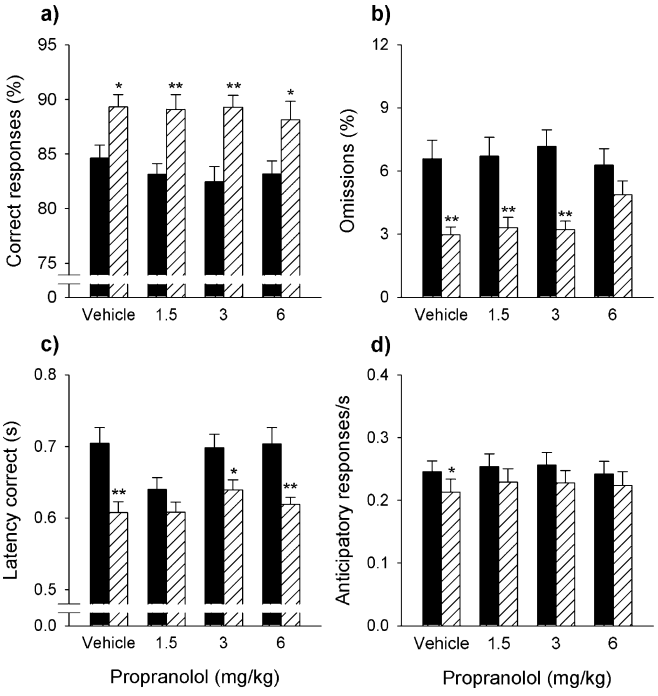

Fig. 3 The effects of systemic injections of propranolol on performance after vehicle (*black bars*) and 0.1 mg/kg of nicotine (*hatched bars*). Bars represent the mean performance (±SEM) of 22 rats in 30-min test sessions with ITI 15 s. Note axis breaks in **a**,**c**. Conditions where nicotine produced a significant difference compared to vehicle are marked (*adjusted $P<0.05$, **adjusted $P<0.01$; paired *t*-tests)

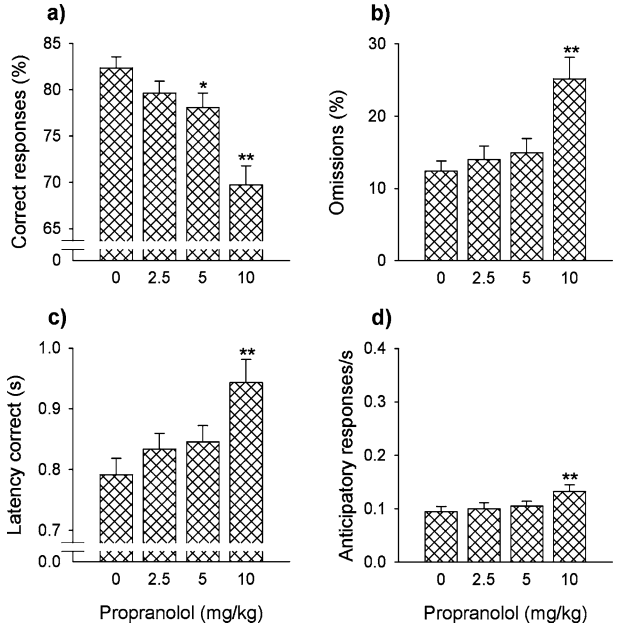

Fig. 2 Dose–response functions of the effects of propranolol on performance under training parameters (ITI 5 s). *Bars* represent the mean performance (±SEM) of 22 rats in 30-min test sessions. Note axis breaks in **a**,**c**. **b** is shown on a different scale than in other experiments. Doses of propranolol that produced significant differences compared to vehicle are marked (*$P<0.05$, **$P<0.01$; Dunnett's test)

Experiment 4: single-dose study on interactions of nicotine with prazosin and propranolol

The aim was to re-test interactions of nicotine with the two adrenoceptors antagonists at optimised doses. Prazosin was tested at a dose below the one that impaired baseline performance in experiment 1. In the preceding experiments, prazosin was not tested against significant effects of nicotine on some measures and thus, antagonism of such effects remained to be investigated. Contrary to experiment 2, experiment 3 indicated that a larger dose of propranolol could be tested without impairing baseline performance. Prazosin (0.5 mg/kg), propranolol (10 mg/kg) and vehicle were tested in the presence of nicotine (0.1 mg/kg) or vehicle in a single sequence of six tests.

Two separate two-factor ANOVA were performed on the data, one with nicotine and prazosin and one with nicotine and propranolol as within-subject factors. As can

be seen from Fig. 4a, prazosin had no effect on response accuracy or on the nicotine-induced increase therein. Accordingly, neither the main effect of prazosin nor the prazosin×nicotine interaction was significant for this measure. Prazosin appeared to increase omission errors and reduce anticipatory responding both in the presence and absence of nicotine (Fig. 4b,d), giving rise to significant main effects [$F(1,20)>23.6$, $P<0.001$] but there were no prazosin×nicotine interactions on these variables. By contrast, Fig. 4c shows that prazosin reversed the nicotine-induced reduction in response latency without affecting this measure when given alone [interaction $F(1,20)=6.03$, $P<0.05$].

Propranolol, even when tested at the larger dose that had profoundly impaired performance in experiment 2, did not display any such effects in the absence of nicotine on any measure. However, it reduced the nicotine-induced increase in response accuracy, as confirmed by a significant propranolol×nicotine interaction [$F(1,20)=10.5$, $P<0.01$]. Propranolol also appeared to weaken the reduction in omission errors and response latency by nicotine, but this was not confirmed by significant interactions. The apparent decrease in anticipatory responding by propranolol was not supported by a significant main effect. In its presence, nicotine did not decrease anticipatory responding as it did in the absence of propranolol, resulting in a significant interaction [$F(1,20)=36.3$, $P<0.001$].

Experiment 5: influence of ITI value on effects of propranolol

Before conducting the final interaction studies with propranolol, it was important to establish if its lack of effect on baseline performance in experiments 3 and 4 (as compared with the impairments observed in experiment 2) was due to tolerance or the difference in task parameters employed. Over four sessions, propranolol (10 mg/kg) and vehicle were tested under both ITI 5 s and 15 s.

Propranolol caused large impairments in accuracy, omissions and response latency when tested with ITI 5 s but not with 15 s (Fig. 5). This was supported by significant propranolol × ITI interactions on accuracy [$F(1,19)=14.8$, $P<0.01$] and omission errors [$F(1,19)=6.37$, $P<0.05$], although not on the latency in two-factor ANOVA for repeated measures. Effect sizes of propranolol with ITI 5 s were similar to those in experiment 2, thus there was no indication of tolerance. The propranolol×ITI interaction was significant also for anticipatory responding [$F(1,19)=6.17$, $P<0.05$]; the slight increase by propranolol under ITI 5 s would have been in accordance with results from experiment 2 but did not reach significance under either ITI.

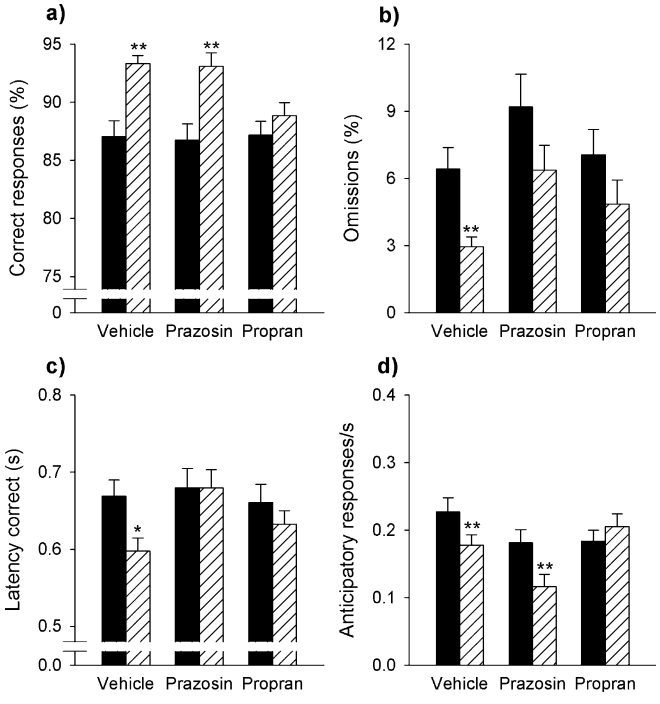

Fig. 4 The effects of systemic injections of 0.5 mg/kg prazosin or 10 mg/kg propranolol on performance after vehicle (*black bars*) and 0.1 mg/kg nicotine (*hatched bars*). Bars represent the mean (±SEM) performance of 21 rats in 30-min test sessions with ITI 15 s. Note axis breaks in **a**,**c**. Conditions where nicotine produced a significant difference compared to vehicle are marked (*adjusted $P<0.05$, **adjusted $P<0.01$; paired *t*-tests)

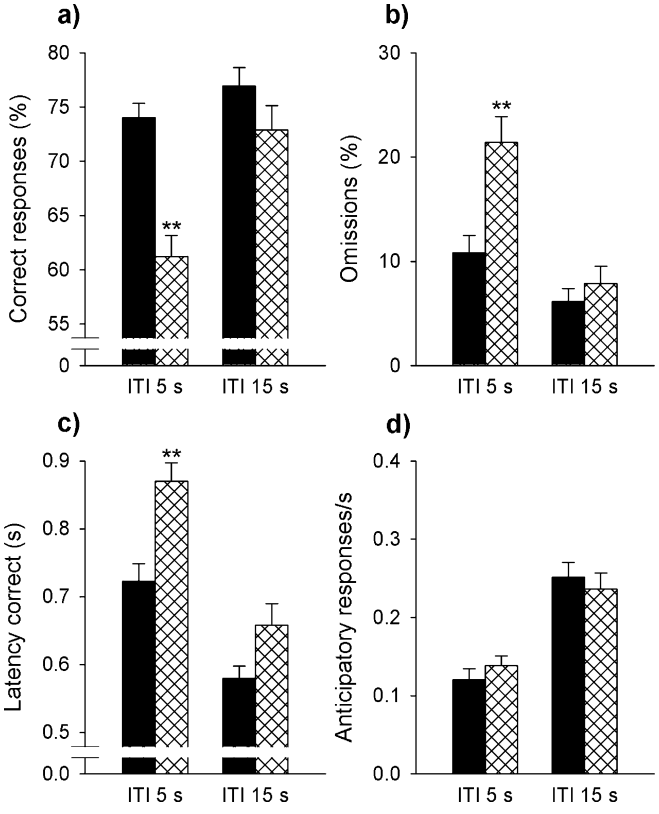

Fig. 5 The effects of 10 mg/kg propranolol (*cross-hatched bars*) as compared with vehicle (*black bars*) on performance of 30-min test sessions with ITI 5 s or 15 s. Bars represent the mean (±SEM) performance of 22 rats. Note axis breaks in **a**,**c**. **a**–**c** are shown on a different scale than in other experiments. Conditions where propranolol produced a significant difference compared to vehicle are marked (*adjusted $P<0.05$, **adjusted $P<0.01$; paired *t*-tests)

Experiment 6: effects of propranolol after repeated daily administrations of nicotine

In experiment 4, propranolol reversed effects of nicotine on accuracy and anticipatory responding that were opposite in direction. In view of the negative relationship between these measures (Hahn et al. 2002a), one interaction may have been secondary to the other. It was desirable to replicate antagonism of the effect of nicotine on stimulus detection by propranolol under conditions where nicotine had no effect on anticipatory responding. With repeated exposure, tolerance develops to nicotine-induced decreases in this measure but to none of its performance enhancing effects (Hahn and Stolerman 2002). Thus, in the 2 weeks preceding experiment 6, rats were injected with 0.4 mg/kg nicotine 2 h after each training session. Propranolol (0.0 mg/kg and 10 mg/kg) was tested against nicotine (0.2 mg/kg) and vehicle both under ITI 5 s and 15 s over a sequence of eight tests. A larger dose of nicotine was chosen because less disruptive effects were expected after the chronic administration of nicotine.

In the absence of nicotine, trends occurred resembling the interaction of propranolol with ITI in experiment 5 (data not shown). However, this was not reflected by significant three-way interactions or interactions of propranolol with ITI on any measure in three-factor ANOVA with propranolol, nicotine and ITI as within-subject factors. Figure 6 therefore presents the effects of propranolol and nicotine collapsed over both ITI values. Nicotine improved and propranolol impaired performance on accuracy, omissions and latency as supported by significant main effects [$F(1,20)>10.4$, $P<0.01$ in all cases]. As in experiment 4, propranolol weakened the effects of nicotine on accuracy, as confirmed by a significant interaction for this measure [$F(1,20)=5.24$, $P<0.05$] but not for any of the other indices. There were no significant main effects or interactions on anticipatory responding, suggesting that tolerance had developed to the effects of nicotine on this measure.

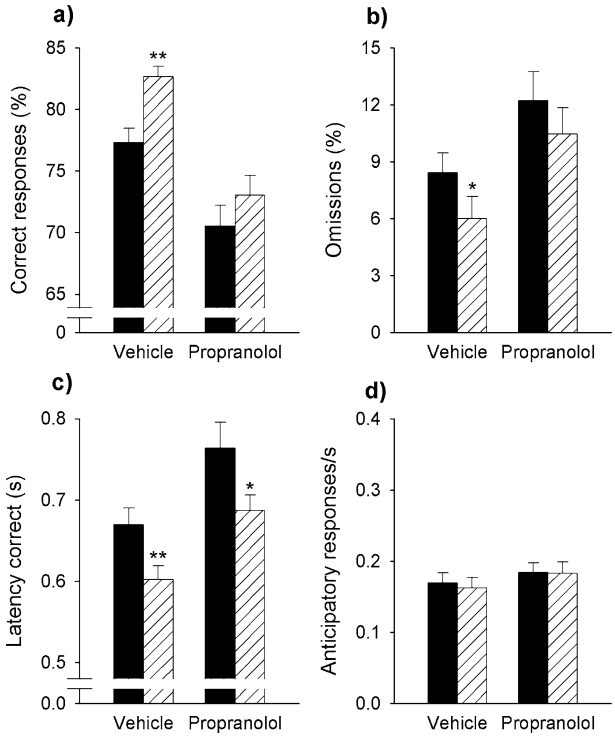

Fig. 6 The effects of systemic injections of propranolol (10 mg/kg) on performance after vehicle (*black bars*) and 0.1 mg/kg of nicotine (*hatched bars*). Twenty-one rats were tested twice in 30-min sessions, once with ITI 5 and once with ITI 15 s. Bars thus represent the mean (±SEM) of 42 observations. Note axis breaks in **a**,**c**. **b** is shown on a different scale than in other experiments. Conditions where nicotine produced a significant difference compared to vehicle are marked (*adjusted $P<0.05$, **adjusted $P<0.01$; paired *t*-tests)

Discussion

The present series of experiments demonstrated differential modulation of the effects of nicotine in the 5-CSRTT by α-adrenoceptor and β-adrenoceptor antagonism. The pattern of effects emerging from these experiments suggests that activation of α-adrenoceptors may contribute to the effects of nicotine on response speed while β-adrenoceptors may be involved in mediating its beneficial effects on signal detection and other attentional functions.

The α_1 (and $\alpha_{2B \text{ and } C}$) adrenoceptor antagonist prazosin weakened the effects of nicotine on omission errors in experiment 1, but this effect was restricted to a dose (1 mg/kg) that also profoundly reduced anticipatory responding in the presence of nicotine. As stressed by Robbins (2002), effects of drugs or lesions on different response indices need to be interpreted in relation to each other to be meaningful. As such, the fact that responding was reduced by prazosin plus nicotine both in the presence and absence of target stimuli points towards non-specific response disruption rather then antagonism of improvements by nicotine. Indeed, the same dose of prazosin reduced spontaneous rearing (Mathe et al. 1996), and the presence of nicotine may have enhanced such motor inhibition.

By contrast, reversal of the nicotine-induced reduction in response latency in experiment 4 occurred at a dose of prazosin (0.5 mg/kg) that had no effect on the latency measure by itself and in the absence of modulation of other nicotine effects. This specificity allows the suggestion that α_1-adrenoceptors are involved in mediating response speed-enhancing effects of nicotine, which would conform to their suggested role in locomotor activity and arousal (Sirvio and MacDonald 1999). In a previous study, the same interaction on response latency was observed between nicotine and the D_2-type dopamine receptor antagonist raclopride, suggesting that the dopamine-releasing action of nicotine is involved in mediating increased response speed (Hahn et al. 2002b). Interestingly, prazosin (0.5 mg/kg and 1 mg/kg) can inhibit dopamine release in the nucleus accumbens stimulated by amphetamine and MK-801, as well as locomotor stimulation by amphetamine, MK-801, cocaine and morphine (Mathe et al. 1996; Darracq et al. 1998; Drouin et al. 2001, 2002). Thus, reversal of speed-related effects of nicotine

by prazosin may be due to α_1-adrenoceptors exerting a modulatory influence over dopamine-mediated effects of nicotine.

There was no indication that prazosin weakened nicotine-induced improvement in response accuracy. In experiment 1, this may have been due to this effect of nicotine being weak and non-significant. In experiment 4, however, the increase in accuracy was robust, and prazosin did not modulate it at a dose that reversed effects of nicotine on another measure (latency). Thus, activation of α-adrenoceptors may not be critical for enhanced stimulus detection by nicotine.

By contrast, the β-adrenocept or antagonist propranolol weakened the effects of nicotine on omission errors (experiment 3, at 6 mg/kg) and on response accuracy (experiment 4 and 6, at 10 mg/kg), but never on response latency. Changes in omission errors can reflect changes in stimulus detection, but also in response rate. Modulation of this measure in experiment 3, however, was not accompanied by other effects of propranolol that would point toward rate-reduction, and even a larger dose than tested in this experiment only minimally decreased anticipatory responding at ITI 15 s. The fact that propranolol antagonised the effects of nicotine on response accuracy in other experiments further supports interpreting this interaction on omissions as a reversal of enhanced stimulus detection. Mediation of this effect by β-receptors would conform with the suggestion that they enhance cortical responses to prolonged depolarisation such as in sensory-evoked responses (McCormick et al. 1991).

Modulation of response accuracy, being a measure of response choice, can never be explained by rate-related or speed-related effects per se. However, interpretative problems arise when improvements in accuracy coincide with decreases in anticipatory responding or vice versa. Inverse correlations between these two measures were found (Hahn et al. 2002a). Anticipatory responses occur largely in the second prior to stimulus onset (Blondel et al. 2000) and are likely to interfere with appropriate visual search strategies of the widely spaced array of target locations. Decreases in anticipatory responding can reduce such behavioural interference. Such decreases can reflect better focusing on task demands (resulting improvements in accuracy may then still be considered as attentional enhancement), but are often due to sedative, ataxic or other noxious side effects of drugs. Any resulting "improvement" in accuracy would then constitute an artefact. Related interpretative pitfalls arose in experiments 1 and 4.

In experiment 1, the largest tested dose of prazosin (1 mg/kg) appeared to facilitate improvements in response accuracy by nicotine, and this was also the only dose to decrease anticipatory responding in the presence of nicotine. The magnitude of this decrease was more suggestive of disruptive side effects of this drug combination then of specific cognitive modulation. Effects of this treatment condition on omission errors support this interpretation. Experiment 1 is likely to constitute an example of response-depressant effects of a pharmacological manipulation causing an "artificial" increase in accuracy. This can lead to interpretative errors in neuropharmacological studies employing the 5-CSRTT.

In experiment 4, propranolol reversed the nicotine-induced increase in accuracy while having no effect on this measure by itself. However, propranolol also abolished the decrease in anticipatory responding by nicotine that may have augmented the nicotine effect on accuracy in the absence of propranolol. While improvements in accuracy can occur even with concomitant increases in anticipatory responding (e.g. Hahn et al. 2002a), it is unknown if and to what degree reductions in anticipatory responding by nicotine can contribute to its effect on accuracy, and if this reflects better attentional focusing or effects of lesser specificity and interest. The absence of disruptive effects on other measures may point toward the former explanation. However, it was desirable to replicate the interaction on accuracy under conditions unconfounded by a parallel interaction on anticipatory responding. When tolerance had developed to nicotine-induced reductions in this measure, propranolol still antagonised the increase in accuracy by nicotine. In addition to results from experiment 3, this offers a solid basis for the suggestion that β-adrenoceptors at least partially mediate enhancement by nicotine in stimulus detection.

Both prazosin and propranolol impaired baseline performance at their respective largest dose tested. For propranolol, such impairments were restricted to tests performed with ITI 5 s as opposed to 15 s. The different sensitivities of these test parameters to impairment by propranolol may reflect involvement of β-adrenoceptors in specific attentional functions. Performing the 5-CSRTT at ITI 5 s requires fast attentional reorienting, especially in the present task version where new ITIs were initiated by responses to stimuli rather than reward collection. Thus, within 5 s a rat had to turn around, collect the reward, turn again, resume visual screening and be prepared to respond to the next stimulus. Performance under such conditions depends on the ability to rapidly reallocate attention, both spatially and from food consumption back to stimulus detection and response requirements. Nicotine has been reported to facilitate rapid attentional shifts in space (Witte et al. 1997; Phillips et al. 2000; Stewart et al. 2001), and mediation of its effects in the 5-CSRTT by β-adrenoceptors may reflect modulation of such aspects of attention. Strong dependence of performance at ITI 5 s on NA neurotransmission would also conform to the suggested role of NA in maintaining behavioural organisation under conditions of non-specific behavioural activation (Carli et al. 1983; Cole and Robbins 1987, 1992).

Hypotensive effects of prazosin and propranolol are unlikely to account for their performance-impairing effects in the 5-CSRTT. Such changes could conceivably decrease response rate and speed (which would be incompatible with the observed increase in anticipatory responding by propranolol under ITI 5 s), but impairment in the accuracy of response choice would be difficult to explain. In rats, prazosin caused only minimal reductions in blood pressure

at a dose (3.15 mg/kg SC) 3 times larger than the maximum dose employed here (Sommermeyer et al. 1995). Propranolol at 2 mg/kg intravenously or 10 mg/kg IP as in the present study did not lower arterial blood pressure of rats (Kittner et al. 1991; Polio et al. 1993).

The present study provides evidence that activation of α-adrenoceptors may contribute to the effects of nicotine on response speed, and activation of β-adrenoceptors to its beneficial effects on stimulus detection. Other secondary neurotransmitter systems may additionally be involved, but the present results already have implications affecting the search for more selective nicotinic agonists for clinical use as attentional enhancers. The ability to upregulate central NA release and activate β-adrenoceptors may be a crucial aspect of effective compounds. Previous studies suggested that the potential to increase dopamine neurotransmission may not be critical for this sought-after effect (Hahn et al. 2002b, 2003a). Such dissociation of neurotransmitter systems involved may aid the process of narrowing down subtypes of the nicotinic receptor to be targeted. Further studies aimed at identifying critical brain regions (e.g. among those expressing β-adrenoceptors) have the potential for taking this process further. As such, the medial prefrontal cortex was identified as a promising target site (Hahn et al. 2003b).

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